No. 36.

(NEW SERIES.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

OBSERVATIONS ON RABIES:

WITH SPECIAL REFERENCE TO AN ATROPHIC FORM OF THE DISEASE OCCURRING IN ANIMALS.

BY

MAJOR G. LAMB, M.D., I.M.S.

AND

CAPTAIN A. G. McKENDRICK, M.B., I.M.S.

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on rabbits in the laboratory it varies much in virulence according to the animal from which it is obtained. The term "fixed virus" is used to denote a virus which by passage through a series of animals has become of such a strength that the incubation period of the disease after subdural inoculation of the virus is practically always the same and cannot be shortened by further passages. As a rule this term is applied to a virus which has become of definitely short incubation period by passage through rabbits, but, as we shall see, we may also have a fixed dog virus, that is to say, a virus which has become fixed by passage through dogs.

Also, it is to be noted that, as the rabies virus is principally found in the central nervous system of animals dead of the disease, it has come about that the term "virus" is sometimes used to denote the central nervous system or portions thereof containing the virus. Thus for the sake of brevity we often talk of an emulsion of virus, when we really mean an emulsion of a portion of the central nervous system containing the virus. No ambiguity, however, has been allowed to occur owing to this use of the word.

II.—Passage of the virus of the street through the dog.

Until quite recently it has been generally held that by passage through certain species of animals, *e.g.*, the monkey and the dog, rabies virus so lost its virulence as ultimately to become innocuous, and, on the other hand, that passage through other species, *e. g.*, the rabbit, exalted the virulence. This supposition was Pasteur's original idea and, supported by the experiments of several other workers, it has been more or less generally accepted. Further, as a consequence of this hypothesis, it has been postulated that in nature the virus, as commonly found in the canine tribe, must occasionally pass through another species of animal in order to retain its virulence. It has been conjectured that the cat or the rat was this other species.

Recently, however, Marie (1907) has made two series of observations on this subject and has obtained somewhat different results. Starting originally with a dog virus of the street and a virus of human origin, he has made in dogs by subdural inoculations 12 passages with the former and 15 passages with the latter. Not only did the virus never fail to give furious rabies but its virulence was so increased after 6 or 7 passages as to give an incubation period of 8 or 9 days. Blasi and Travali (1894) working with rats and cats had previously obtained similar results. There was no attenuation of the virus. On the other hand it was found that the incubation period became very short, shorter than by passage through the rabbit.

The experiments which we have made on this question were carried out on the dog. The original virus was contained in the central nervous system of

a dog which had died of furious rabies in Meerut. When this virus was inoculated subdurally into a rabbit the incubation period of the disease was 11 days. Before the first passage was made the virus had been kept in glycerine for three weeks.

A reference to Table 1 of the protocols will show that in all 13 passages were made, the first three by subdural and the remaining ten by intraocular inoculations. All the dogs developed furious rabies and died very shortly after the onset of symptoms.

The points, worthy of note, which emerge from a study of the table, are as follows—

The first three passages reduced the incubation period from 11 to 8 days. It has already been mentioned that in these passages the inoculations were made subdurally.

In the first passage made by intraocular injection the incubation period was lengthened from 8 to 13 days. Further, it would appear that while the infection was easily carried on from dog to dog when the intraocular method was used, the incubation period was somewhat irregular in succeeding passages. While this is so, there was on the whole a gradual shortening, that is to say, a gradual increase of virulence. Thus the incubation period between the 4th and 9th passage was shortened from 13 to 8 days, although the 7th dog had an incubation period of 20 days. The dogs of the last two passages again had somewhat prolonged incubation periods, but these were not due to a diminution in virulence. This is shown not to be the case, as a rabbit inoculated subdurally with an emulsion of the medulla of the dog of the last passage developed rabies in 7 days, that is to say, the usual incubation period of a virus which has become fixed by passage through the rabbit. We had, therefore, increased the virulence of the dog virus of the street for the rabbit, obtaining a virus which, in this respect at least, did not differ from ordinary fixed rabbit virus. The bearing of this fact will be referred to later on.

Finally, we have to draw attention to the results of a microscopic examination which was made to determine the presence or absence of Negri bodies in certain portions of the central nervous system of all the dogs which died in the course of these passages. The technique employed was to fix small pieces of the tissues in Zenker's fluid, to cut in paraffin and to stain by Mann's or Unna's method.

In the dog of the first passage Negri bodies were found to be abundant in the horn of Ammon, plentiful in the cerebral cortex, and few in the cerebellum.

In the dogs of the next few passages they were also easily demonstrated, especially in the hippocampus major. In the dog of the 5th passage, there was doubt as to their presence and if they were present they were very few in

number and small in size. In the dog of the 6th passage they could not be found nor were they demonstrable in any of the succeeding animals, although careful search was made in every instance through well stained specimens.

It would appear, therefore, that the same thing happens when the virus of the street is passed through dogs as occurs when it is passed through rabbits. We have searched for Negri bodies, but without success, in the central nervous system of the passage fixed virus rabbits. Further, Schiffmann has found that by passing the virus of the street through rabbits, Negri bodies soon disappear. He looks upon this disappearance as a means of deciding whether one is dealing with a definitely fixed virus, or not.

SUMMARY.

A virus originally obtained from a dog in the street was passed through a series of 13 dogs, by subdural inoculation or by intraocular injection. An increase of virulence resulted. The virulence of the virus was also increased for the rabbit.

Negri bodies were demonstrable in certain cells of the central nervous system of the dogs of the first few passages, but not in the same cells of the animals of the later passages.

III.—A peculiar form of rabies seen in rabbits and dogs.

During the last few years in the course of the routine work of the Institute it has frequently been observed that a rabbit, which had received a subdural inoculation of street virus remained in good health for a time. It then gradually became emaciated and died without developing any of the symptoms of rabies which are usually seen in the rabbit, namely, paralysis, tremor, etc. In most instances the emaciation was of slow progress, the disease lasting a month or more. In other cases, however, the wasting was observed to begin very soon after the infection and to run a very rapid course, so that death took place within 10 days of the inoculation. At first these rabbits were not considered to be suffering from rabies. It was thought that the symptoms might be due to the toxicity of the foreign nerve material which had been introduced. On further investigation, however, it was found that rabbits injected subdurally with emulsions of the medulla of these emaciated animals developed typical rabies. It was also found that in some instances, before the usual symptoms of rabies appeared, two or more passages had to be made, the rabbit of the first passage developing symptoms similar to those seen in the ori-

ginal animal, that is to say, becoming emaciated and dying without showing any tremor or paralysis. In Protocol II several examples of this type of rabies have been set forth.

The first two observations (Table 2) relate to rabbits which had received subcutaneously a dose of fixed rabbit virus.

In the first example (*a*) slow progressive emaciation was observed followed by death 58 days after the inoculation. The passage rabbit from this animal also became gradually thinner without developing paralysis, and died 27 days after infection. The disease, therefore, had run a more rapid course than was the case with the first animal. The second passage rabbit showed typical symptoms of rabies,—tremor, paralysis, etc., on the 20th day and died on the 21st day. It did not become emaciated. In the second example (*b*) the emaciation began soon after the inoculation and ran a fairly rapid course, death taking place in 22 days. The passage animal showed marked paralysis and tremor on the 7th day.

In a second group of observations, examples of which are given in Table 3, the inoculations were made subdurally with emulsions of the brain of dogs which had died of suspected rabies, that is to say, with the virus of the street. In each instance the pieces of brain had been in glycerine for a few days. Three cases are recorded in the protocols. In one case the rabbit lived 57 days and in another 17 days. The third case is remarkable, inasmuch as the emaciation began immediately after the infection and ran a very rapid course, death taking place 9 days after the subdural inoculation. The passage rabbit from each of these original animals showed definite and typical symptoms of rabies between the 11th and 13th days.

In the examples of this disease to which we have drawn attention above, it is seen that death has always resulted. In some instances, however, a similar condition has been observed, but the animals have ultimately recovered. They have undergone progressive emaciation and become very weak, exactly as we have already described. But instead of death taking place, the animal after a time has gradually picked up and ultimately completely recovered. We may cite the following cases—

On the 11th November, 1907, a rabbit was inoculated subcutaneously with 0·01 gramme of fixed rabbit virus. A month afterwards it was observed to be wasting, and by the end of December it was noted to have become much emaciated and very weak. After a time it began to recover and by July 1908 it was found to be fat and sleek.

A second example is that of a rabbit inoculated subdurally with an emulsion of the medulla of a rabbit which had died very emaciated about two months after the subcutaneous inoculation of fixed virus. This animal infected on the 29th

January, 1908, was noted by the middle of March to be very thin and weak. It then gradually got better and by July was plump and well.

It is impossible to say whether these were true infections of rabies or not. Neither of the above two rabbits was immune to a subsequent subdural infection of fixed rabbit virus. They were inoculated subdurally each receiving 0·2 c.c. of a thick emulsion of fixed rabbit virus. They developed hydrophobia after an incubation period each of 7 days and died on the 12th day.

Finally, the question arises if this form of rabies, characterised chiefly by progressive emaciation, occurs in nature amongst dogs or other animals. We have had reported to us several cases of sickness in dogs, in which the symptoms closely resembled those described above in rabbits. In some of these cases the experimental proof of rabies was obtained after the death of the animal, but only one dog which might be said to have suffered from the type of disease we are now considering, has come under our personal observation. Its history was as follows—

It was bitten on or about the 12th February, 1908, by a dog which was experimentally proved to be rabid. There were two wounds on the face: these were cauterised. About the 7th March—that is to say, from 3 to 4 weeks after being bitten, the animal was noticed to be ill and to have become thinner. The emaciation progressed and on the 1st April was well marked. No paralysis was noticed on this date. On the 3rd of April the animal refused food for the first time. Next day it howled incessantly and the jaw was then noticed to have dropped. It died on the 5th April. Negri bodies, numerous but of small size, were demonstrable in the cells of the hippocampus major. A rabbit inoculated subdurally with an emulsion of the medulla developed marked paresis by the 12th day and died on the 13th day.

SUMMARY.

Several cases of a form of rabies in the rabbit, in which progressive emaciation is the chief symptom, are described. This type has been observed to follow both subcutaneous inoculation of fixed rabbit virus and subdural injection of street virus.

The disease generally runs a more or less chronic course but may be comparatively rapid. The fact that this condition is true rabies has been experimentally demonstrated. On sub-passage from animals dead of this form of the disease typical symptoms of rabies in the rabbit have developed.

Rabbits showing symptoms of progressive emaciation after injection of rabies virus have been observed to recover completely.

Finally this type of the disease has been observed in a dog infected in nature.

IV.—The susceptibility of various animals towards fixed rabbit virus.

It has long been known that, although it is easy to infect animals with rabies by subdural, intraocular or even intramuscular inoculation of fixed rabbit virus, certain species display a marked degree of insusceptibility to the subcutaneous injection of even large quantities of this virus.

Most observers have experienced considerable difficulty in infecting dogs by the subcutaneous inoculation of either fixed or street virus. Thus Bardach (1888) was able to infect only 1 out of 22 dogs and Helman (1889) obtained similar results. The latter also failed to infect monkeys by subcutaneous injections of fixed virus.

Most workers have found that rabbits were more easily infected by the subcutaneous channel than dogs.

Finally, it is generally believed that fixed rabbit virus is harmless to man when injected subcutaneously and in this belief Nitsch (1905) injected himself with 3 c.c. of an emulsion of the fresh cord of a passage rabbit.

We may now ask ourselves the question upon what factor does this comparative immunity of some animals to subcutaneous inoculation depend. Helman (1889) believes that rabies virus produces infection only when introduced directly into nervous tissues. It might also be suggested, as Nitsch evidently believes, that the passage of the virus through the rabbit may so modify it as to render it incapable of infecting certain other species, such as man, monkey, etc., when injected subcutaneously, that, in short, there is some fundamental difference between the virus of the dog and the virus of the rabbit—a difference similar to that which is found in the modification, as regards its infectivity for man, which small-pox virus undergoes when passed through the calf.

Although we have not been able to obtain a definite answer to this question, we have several series of observations to record which bear upon it. These observations consist, (1) of experiments made with the view of testing the susceptibility of three different species of mammals to the subcutaneous inoculation of fixed rabbit virus; (2) of experiments made with the view of comparing in a single species, namely, the guinea pig, and by subcutaneous inoculation, the infectivity of fixed rabbit virus with that of a dog virus, and (3) of observations on the susceptibility of monkeys to fixed rabbit virus inoculated by different channels.

A.—Susceptibility of guinea pigs, rabbits and monkeys to subcutaneous inoculation of fixed rabbit virus.

For these experiments a portion of the central nervous system of one of the ordinary passage rabbits was used. The passage was between the 225th and

230th from the dog, the incubation period in a rabbit after injection subdurally being, as a rule, six days, death taking place on the 10th day.

From a portion of the central nervous system of the rabbit an emulsion of definite strength was prepared and from this emulsion various dilutions were made as required. The amount of emulsion injected was always made up to 5 c. c. Great care was taken that the muscles were not penetrated and that the injections were really made into the subcutaneous tissues.

The results of these experiments are set forth in Tables 4, 5 and 6 of the protocols.

Table 4 refers to guinea pigs, which, it will be seen, readily develop rabies even when a very small quantity of virus is injected. With large amounts of virus, however, the incubation period is on an average somewhat shorter than with small quantities.

Table 5 refers to rabbits, which received the same amounts of virus as were used in the case of the guinea-pig experiments.

It is first to be noted that rabbits can be infected fairly easily by subcutaneous injection of this virus, 14 out of 18 in the present series developing the disease. Secondly, they would appear to be as susceptible to small amounts of virus as to large quantities when the inoculations are made under the skin. Thirdly, it is interesting to observe that the disease which follows subcutaneous injection into rabbits is, as a rule, a more or less chronic one, without any of the usual symptoms of rabies. In some instances, *e.g.*, Rabbits 1, 10, 11 and 18, the animals showed tremor and paresis at the end of a definite incubation period, but, as a rule, the onset of the disease could not be determined and death, preceded by progressive emaciation, followed a considerable time after the infection. We have already referred to this type of the disease and have endeavoured to show that it is true rabies. Finally, a study of the table will show that it cannot be said that the duration of the disease was at all proportional to the amount of virus injected.

The last series of experiments refers to monkeys.

In Table 6 are put down the details relating to three monkeys which were injected subcutaneously, each with a considerable quantity of fixed rabbit virus. Two of these animals died of rabies, one after 48 days and the other after 76 days, that is to say, in each instance after a prolonged period of latency.

It is interesting to note, *vide* Table 6, that although these monkeys were injected with fixed virus which gave an incubation period of 6 days when injected subdurally into rabbits, two rabbits inoculated subdurally with thick emulsions of the medullas of the monkeys did not develop the disease till after 18 and 31 days respectively. Both these rabbits showed symptoms typical of rabies. Therefore, by a single passage through the monkey, infected subcu-

taneously, the incubation period of the fixed rabbit virus had been greatly prolonged for the rabbit. This observation is of importance in connection with the finding that the virus taken from the central nervous system of men who have died of rabies in spite of anti-rabic treatment has nearly always given on subdural inoculation into rabbits an incubation period considerably longer than that of the fixed rabbit virus. On this evidence it has been concluded that the virus which has caused the death of these persons is the dog virus of the street, inoculated at the time of the bite, and not the fixed rabbit virus used for the preparation of the vaccine. Our present observations in the monkey show that this method of argument is not justifiable.

B.—Comparison of the susceptibility of guinea-pigs to fixed rabbit virus and to virulent dog virus, both being injected subcutaneously.

As the guinea-pig is the most susceptible animal to subcutaneous injection of a virus which has become fixed by passage through the rabbit, the guinea-pig was now used to see if there was any difference in this respect between this virus and a virus obtained from a dog and virulent for that animal, which had never been passed through the rabbit.

The data which refer to the susceptibility of guinea-pigs towards fixed rabbit virus have already been put forward in Table 4. Similar data concerning the dog virus are set forth in Table 9. The virus was originally obtained from an animal which had died of furious rabies in Meerut; after having been kept in glycerine for several days it gave an incubation period of eleven days when inoculated subdurally into a rabbit. The virus was first passed through two dogs by subdural inoculation. Both dogs died of furious rabies, the first after twelve days and the second after ten days. The medulla of the second dog was now used to prepare the emulsion for inoculation into guinea-pigs, the same technique being employed as has already been described in connection with the inoculation of rabbit fixed virus into guinea-pigs. From a comparison of the two tables, namely, Nos. 4 and 9, we can only conclude that the guinea-pig is as susceptible to fixed rabbit virus as to a virulent dog virus when the inoculations are made subcutaneously. It would appear, therefore, that passage through the rabbit does not modify to any appreciable degree in the virus as far as its power of infecting guinea-pigs is concerned.

C.—Susceptibility of monkeys to fixed rabbit virus.

We have already seen that it is possible to infect monkeys with fixed rabbit virus when injected subcutaneously in comparatively large quantity. We

have now to add to this observation two other series of experiments which have reference to the susceptibility of monkeys towards fixed rabbit virus.

In one series (Table 7) three monkeys were inoculated intraperitoneally with 0·5 gramme of fixed virus. Two of these animals developed rabies with a comparatively short incubation period, namely, 15 and 16 days respectively. In the other series (Table 8) three monkeys were inoculated subdurally with 0·003 gramme (0·3 cc. of a 1 per cent. emulsion) of fixed rabbit virus, which gave in the rabbit after subdural inoculation an incubation period of 6 days. It is seen from the table that all these three monkeys developed rabies, with incubation periods of 8 and 9 and 10 days respectively.

It would appear, then, that monkeys are susceptible by all ordinary channels to a virus which is separated from the dog by many passages through the rabbit. Dog virus does not, therefore, become modified as regards its infectivity for monkeys, by passage through the rabbit, as tested by these methods.

We have already seen, that there are certain facts which suggest that the virus obtained by passage through the rabbit is, at least qualitatively, the same thing as the virus of the dog. We have now brought forward further facts which favour this view. It is necessary, therefore, before quitting this part of our subject, to bring together the evidence which supports this hypothesis. It is stated as follows—

1. By passage of the ordinary street virus through the dog, subdural or intraocular methods being employed, its virulence for the dog is increased. At the same time its virulence for the rabbit is exalted, so that it ultimately produces the disease in rabbits after the same incubation period as does fixed rabbit virus.

It is to be remembered that dogs are quite as susceptible as rabbits to the subdural inoculation of fixed rabbit virus.

2. Negri bodies are found in the central nervous system of practically every dog which dies of rabies in nature. On passage of this virus through the dog by subdural or intraocular injection, these bodies after a few passages can no longer be demonstrated. The same phenomenon has been observed in passing street virus through the rabbit by subdural inoculation.

3. No difference is observed between fixed rabbit virus and fixed dog virus, as tested by subcutaneous inoculation into guinea-pigs.

4. Monkeys have been shown to be susceptible to fixed rabbit virus when injected subdurally, intraperitoneally or subcutaneously.

If, then, the rabbit virus has the same properties as the dog virus and has not become qualitatively modified by passing through the former animal, we are almost forced to accept Helman's hypothesis on the problem of the relative insusceptibility of certain animals to subcutaneous inoculation: that it must be a question of the site where, and the manner in which, the virus is deposited in

the subcutaneous tissues. In connection with this hypothesis attention may be drawn to the fact that fine emulsions of the medulla containing fixed virus, especially if dilute, may very soon completely lose their infective power when kept at 37°C. (*vide* Table 12). It would appear as if separation from living nervous tissue was sufficient to bring about the rapid death of the virus when kept at body temperature.

SUMMARY.

1. It has been shown that it is comparatively easy to infect guinea-pigs by the subcutaneous injection of fixed rabbit virus even in small quantity. They are equally susceptible to subcutaneous inoculation of virulent dog virus.

2. Rabbits also are susceptible to injections of fixed rabbit virus under the skin. The disease which results is often of a more or less chronic nature, mainly characterised by progressive emaciation without any definite paralysis.

3. Monkeys also can be infected by subcutaneous injection of fixed rabbit virus. The incubation period is much prolonged in these cases. It was found that passage through a single monkey may greatly prolong the incubation period of fixed rabbit virus for rabbits.

Monkeys are also susceptible to the subdural and intraperitoneal injection of fixed rabbit virus. The incubation period after subdural infection is only slightly longer than in the case of the rabbit similarly infected.

4. Certain observations are put forward which point to the conclusion that a virus fixed by passage through the rabbit has the same properties and is identical in character with street virus, exalted in virulence by passage through the dog.

V.—An attempt to immunise monkeys by means of single doses of fixed rabbit virus injected subcutaneously.

An attempt was made in two series of experiments to immunise monkeys by means of a single dose of fixed rabbit virus given subcutaneously As we have already shown that these animals may develop rabies after the injection under the skin of a large quantity of this virus, the incubation period, however, being greatly prolonged, it was hardly to be expected that any immunity would result. Our results show this to be the case.

In the first series of observations (Table 10) six monkeys each received a considerable amount, namely, 0·5 gramme of fixed rabbit virus.

Twenty-three days after this injection each monkey along with two untreated controls received a subdural inoculation of a small amount of fixed rabbit virus. As will be seen from the table all the monkeys died of rabies, and no appreciable

difference in the incubation periods between the treated and untreated animals could be made out.

In the second series of observations (Table 11) six monkeys each received subcutaneously a different amount of fixed rabbit virus, varying from 0·5 to 0·0015 gramme. Twenty-three days after this inoculation each animal, as well as an untreated control, was injected subdurally with a small amount of fixed rabbit virus. As a result all the monkeys died of rabies and again there was no indication of any one of them having developed even a slight degree of immunity.

SUMMARY.

In the hope that it might be possible to curtail the lengthy course of treatment now in use, attempts were made to immunise monkeys by means of a single injection of fixed rabbit virus under the skin. In one series of experiments the amount of virus injected was varied. The animals were tested twenty-three days after the inoculation by giving subdurally a small amount of fixed rabbit virus. All the monkeys died of rabies and it would seem that no degree of immunity had developed.

VI—Bactericidal properties of the serum of patients taken both during the course of anti-rabic inoculations and after the treatment had been completed.

Several workers—Babes and Cerchez (1891), Babes and Talasescu (1894), Marie (1904-1907), Remlinger (1906-1907), Semple (1908)—have been able to obtain from animals immunised with rabies virus a serum which, they state, destroyed the virus when kept in contact with it "in vitro." Sera of highly immunised animals have been used experimentally with the object of ascertaining if they had any hindering effect on the evolution of the disease when injected either before or after inoculation with the virus. Finally, attempts have been made to immunise animals with a mixture of an immune serum and rabies virus.

In the present communication we do not intend to enter into these questions, but to confine ourselves to the consideration of a number of observations made with the object of finding whether bactericidal properties could be demonstrated in the blood of patients either during anti-rabic treatment or shortly after completion of treatment. In this connection it is to be noted that Kraus and Kreissl (1902) have made some experiments which go to show that anti-bodies towards the rabies virus only appear in the blood of those subjected to anti-rabic inoculations 20 to 22 days after the treatment had ended. On the other hand, Semple (1908) has put forward evidence which in

his opinion shows that these substances could be demonstrated in the blood of patients, as soon as 2 or 3 days after the completion of treatment.

In seeking for a technique which would serve our purpose we were first met with the difficulty which arises from the fact, well known to those who have worked with rabies virus, that emulsions prepared in the same proportions from the nervous systems of different animals injected with fixed virus may vary somewhat in virulence. Further, it has been observed by us that emulsions of rabies virus kept at 37° C soon lose their infective power and that this loss is of a progressive nature. This point is illustrated in the experiments set forth in Table 12. These experiments consisted in keeping two emulsions of rabies virus of different strengths in the incubator at 37° C. and testing their infectivity at varying intervals by subdural inoculation into rabbits. The emulsions of strengths 1 in 200 and 1 in 1,600 were made from the medulla of a rabbit dead in 10 days after the subdural injection of fixed rabbit virus, the test dose being 0·4 c. c. It will be seen from the table that even after having been kept for one hour at 37°C. both emulsions had diminished considerably in infectivity and that the loss was progressive until at the end of 24 hours they were no longer infective.

Special precautions had therefore to be taken in order to guard against these two sources of fallacy. These precautions consisted, first, in making each day for each series of experiments a series of control experiments in which normal salt solution was substituted for the sera which were being tested ; and secondly, in keeping the mixtures only for either 1 or 2 hours at room temperature.

Finally, in order to arrive at a quantitative estimation of the bactericidal power of the sera, if there was any, each was tested against several emulsions of the virus of different strengths. The technique employed was as follows—

The blood of the person to be tested was drawn from a vein by means of a sterile syringe. It was placed in a small conical glass and set aside to clot. The serum was left in contact with the clot for 24 hours.

The rabies virus emulsion was prepared from the medulla of a rabbit which had died from the effects of a subdural inoculation of fixed rabbit virus. Only those rabbits were used which showed paralysis on the 6th day and died on the 10th day after infection. Emulsions of the medulla of varying strengths, as indicated in the tables, were made with sterile salt solution. Equal quantities of these different emulsions and of the sera to be tested were thoroughly mixed and the mixtures were kept at room temperature for either 1 or 2 hours ; 0·4 c. c. of each mixture was then injected subdurally into a rabbit. For each series of serum experiments a series of control experiments with salt solution was made. This consisted in mixing the

various dilutions of the virus with an equal amount of salt solution instead of serum, allowing these mixtures to stand under the same conditions as the serum mixtures and then injecting 0·4 c. c. of each subdurally into a rabbit.

It is perhaps necessary to state that the method of anti-rabic treatment which was used for the patients whose blood was tested was that elaborated by Högyes of Budapest.

Six series of experiments were made. They are briefly stated as follows—

Series 1. (*Table 13*).—With sera of two patients who were still under treatment, which had lasted for 9 and 14 days, respectively.

Series 2. (*Table 14*).—With the pooled sera of 5 patients drawn on the day on which the treatment was completed.

Series 3. (*Table 15*).—With the sera of two patients who had completed treatment one day and five days previously. In this series experiments with a normal human serum were also made.

Series 4. (*Table 16*).—With the sera of two patients drawn 5 days and 10 days respectively after completion of treatment.

Series 5. (*Table 17*).—With the sera of two patients who had completed treatment 10 days and 14 days previously. Experiments with a normal human serum were also made.

Series 6. (*Table 18*).—With the serum of a patient who had completed treatment 20 days previously.

The results of these observations are fully set forth in the protocols. It is, therefore, unnecessary to do more than draw attention to the conclusion which a study of the tables forces upon us, namely, that it was impossible to demonstrate bactericidal properties in any of these sera by the methods employed by us.

Any alteration in serum properties is entirely eclipsed by individual variation in the rabbits, and by errors of dose. It is, however, interesting to point out that if a quantitative method of experimentation had not been employed we might have been misled. Thus, if we take the experiments of series 6 it is seen that both with the dilutions of 1 in 50 and 1 in 150 the incubation period as compared with the controls was delayed two days. It might be argued from this result that a certain amount of virus in the emulsions had been destroyed, the smaller amount left giving a lengthened incubation period. That this conclusion would be erroneous is at once apparent from the results of the experiments with a higher dilution, namely, 1 in 1,500. In this the serum mixture gave an incubation period of 9 days while in the control animal the incubation period was two days longer, namely, 11 days.

Before closing this part of our subject we may in passing draw attention to a number of experiments, the results of which are set forth in Tables

19, 20 and 21 of the protocols. These observations were made in order to ascertain if any bactericidal action towards rabies virus could be demonstrated in the sera of a few normal animals, namely, guinea-pigs, rabbits and monkeys. As a rule the sera were tested against fixed rabbit virus, but in one series with rabbit and monkey sera, dog virus was used. The same quantitative method as we have already described was employed.

A study of the tables will at once show that in no instance could any bactericidal action of the sera be demonstrated.

SUMMARY.

Experiments were carried out with the object of making a quantitative estimation of the bactericidal action, if such existed, of the sera both of patients undergoing anti-rabic treatment and of others who had completed the treatment. Patients were selected at various stages. The earliest had been under treatment for 9 days, and the latest had completed treatment 20 days previously. Our experiments failed to demonstrate the presence of bactericidal action towards rabies virus in any of the sera.

The sera of normal guinea-pigs, rabbits, monkeys and men were tested in the same way. The results were also negative.

Protocols.

No. I.—Passage of rabies virus through the dog.

Table 1.—Showing the result of the passage of the virus of the street through a series of dogs.

The original virus was obtained from a dog which died in Meerut showing typical symptoms of furious rabies. The incubation period of this virus when inoculated subdurally into a rabbit was 11 days.

The virus had been in glycerine for three weeks before the series of passages was begun.

Number of passage.	Method of inoculation.	Incubation period in the dog.	Incubation period in rabbit inoculated subdurally.	Remarks.
Original Dog.	...	...	11 days.	
1	Subdural	11 days		
2	„	10 „		
3	„	8 „		
4	Intraocular	13 days.		
5	„	12 „		
6	„	11 „		
7	„	20 „		
8	„	9 „		
9	„	8 „	8 days	
10	„	16 „	9 „	Piece of brain had been 24 days in glycerine.
11	„	9 „	8 „	
12	„	13 „	12 „	Brain had been 2 days in glycerine before inoculation into rabbit.
13	„	29 „	7 „	

Protocols.

No. II.—A PECULIAR FORM OF RABIES SEEN IN RABBITS AND DOGS.

1.—Rabbits inoculated subcutaneously with fixed rabbit virus.

These rabbits received subcutaneously 5 c. c. of an emulsion of fixed rabbit virus of definite strength. It was the 230th passage from the dog and gave an incubation period of 6 days when injected subdurally into rabbits.

Table 2.—Showing the modification of symptoms and course, by sub-passage, until true rabies is developed.

Animal.	Amount of virus.	Method of injection.	Interval between injection and death.	Remarks.
A. Rabbit 1 (original).	0·0001 gram of medulla.	Subcutaneous.	58 days	Became progressively thinner: no paresis.
Rabbit 2, passage from 1.	Thick emulsion of medulla.	Subdural	27 „	Became progressively thinner: no paresis.
Rabbit 3, passage from 2.	Thick emulsion of medulla.	Subdural	21 days	Marked paralysis: no emaciation.
B. Rabbit 1 (original).	0·01 gram of medulla.	Subcutaneous.	22 „	Rapid wasting: no paralysis.
Rabbit 2, passage from 1.	Thick emulsion of medulla.	Subdural	8 „	Showed marked paralysis on 7th day.

Protocols.

2.—Rabbits inoculated subdurally with virus of the street.

These animals were inoculated subdurally with a thick emulsion of the cerebral cortex of dogs which had died in nature of suspected rabies. The brain had been sent to the laboratory for diagnosis. Before injection it had been kept in glycerine for several days.

Table 3.—Showing the data of three further examples.

Animal.	Amount of virus.	Method of injection.	Interval between injection and death.	Remarks.
		A.—Wardha Dog.		
Rabbit 1 (original) .	Thick emulsion.	Subdural	57 days	Progressive emaciation: no paralysis.
Rabbit 2, passage from 1.	Ditto	Ditto	14 „	Marked paralysis in 13 days.
Rabbit 3, passage from 2.	Ditto	Ditto	16 „	Marked paralysis in 14 days.
		B. Rawalpindi Dog.		
Rabbit 1 (original) .	Ditto	Subdural	9 days	Very rapid emaciation: no paralysis: no tremor.
Rabbit 2, passage from 1.	Ditto	Ditto	14 „	Marked paralysis on 11th day: no emaciation.
		C. Bakloh Dog.		
Rabbit 1 (original) .	Ditto	Subdural	17 days	Rapid progressive emaciation: no paralysis.
Rabbit 2, passage from 1.	Ditto	Ditto	14 „	Paralysis and tremor marked on 12th day: no emaciation.

No. III.—The susceptibility of various animals towards fixed rabbit virus.

A. Table 4.—Subcutaneous injection of fixed rabbit virus into guinea-pigs.

The virus used in this series of experiments was between the 225th and 228th passage from the dog: the incubation period after subdural inoculation into a rabbit was 6 days.

A one per cent. emulsion of the medulla was prepared with sterile normal salt solution. From this emulsion different dilutions were made as required. Each guinea-pig received a definite amount of the virus, the quantity of emulsion being in each case made up to 5 c. c. which were injected in two places in the abdominal region. Care was taken that the muscles were not penetrated.

Protocols.

Animal.	Amount of virus.	*Result.	Remarks.
Guinea-pig 1	0·01 gramme	10 days 11 „	
„ 2	Ditto	13 „ 16 „	
„ 3	Ditto	Remained well	
„ 4	Ditto	12 days 14 „	Six out of eight guinea-pigs developed rabies.
„ 5	Ditto	10 „ 11 „	Average incubation period=11 days. Average period between injection and death=12·5 days.
„ 6	Ditto	9 „ 10 „	
„ 7	Ditto	Remained well	
„ 8	Ditto	12 days 13 „	

* The upper number=Incubation period.
The lower number=Interval between injection and death.

Protocols.

Animal.	Amount of virus.	* Result.	Remarks.
Guinea-pig 9	0·001 gramme	10 days 11 „	
„ 10	Ditto	14 „ 17 „	
„ 11	Ditto	Remained well	Five out of six guinea-pigs died of rabies.
„ 12	Ditto	11 days 15 „	Two showed no definite symptoms except loss of weight. Average incubation period in 3 cases =11·6 days.
„ 13	Ditto	... 10 days	Average period between injection and death in 5 cases=13·4 days.
„ 14	Ditto	... 14 days	
„ 15	0·0001 gramme .	... 41 days	
„ 16	Ditto .	Died from an intercurrent disease.	
„ 17	Ditto .	27 days 29 „	Five out of six guinea-pigs died of rabies.
„ 18	Ditto .	16 „ 17 „	One showed no definite symptoms. Average incubation period in 4 cases= 30 days.
„ 19	Ditto .	18 „ 22 „	Average interval between injection and death=34·6 days.
„ 20	Ditto .	59 „ 64 „	

* The upper number=Incubation period.
The lower number=Interval between injection and death.

B. Table 5.—Subcutaneous injection of fixed rabbit virus into rabbits.

The virus used was the 230th passage from the dog. The incubation period in a rabbit after subdural inoculation was 6 days.

Protocols.

The same technique was used as has been described for the similar series of experiments in guinea-pigs.

Animal.	Amount of virus.	*Result.	Remarks.
Rabbit 1	0·01 gramme	13 days 15 „	Four rabbits out of six died of rabies. Three showed the peculiar emaciating type of the disease. The interval between infection and death varied from 15 to 93 days with a mean of 49·5 days.
„ 2	Ditto	... 68 days	
„ 3	Ditto	... 22 days	
„ 4	Ditto	... 93 days	
„ 5	Ditto	Recovered	
„ 6	Ditto	„	
„ 7	0·001 gramme	... 63 days	Five rabbits out of six died of rabies Three developed the emaciating type of the disease. The interval between infection and death varied from 23 to 95 days, the mean being 54·6 days.
„ 8	Ditto	... 95 days	
„ 9	Ditto	... 52 days	
„ 10	Ditto	20 „ 23 „	
„ 11	Ditto	37 „ 40 „	
„ 12	Ditto	Recovered	

* The upper number = Incubation period.
The lower number = Interval between injection and death.

Protocols.

Animal.	Amount of virus.	*Result.	Remarks.
Rabbit 13	0·0001 gramme	... 84 days	Five rabbits out of six died of rabies. Four developed the chronic emaciating form. The interval between infection and death varied from 21 to 113 days, the mean being 66·4 days.
„ 14	Ditto	Recovered	
„ 15	Ditto	... 58 days	
„ 16	Ditto	... 113 days	
„ 17	Ditto	... 56 days	
„ 18	Ditto	18 „ 21 „	

* The upper number = Incubation period.
The lower number = Interval between injection and death.

C.—Injection of fixed rabbit virus into monkeys.

1. Table 6. Subcutaneous inoculation.

The virus used was the 230th passage from the dog: the incubation period in rabbits after subdural inoculation was 6 days. The mid-brain, cervical cord and part of cerebellum were used for the preparation of the emulsion, the strength of which was 10 per cent. Each animal received 5 c. c. of this emulsion. Care was taken that the muscles were not penetrated.

Animal.	Amount of virus.	Result.	Remarks.
Monkey 1	0·5 gramme	Died after 48 days. Rabies.	Rabbit inoculated subdurally with emulsion of medulla showed marked paresis on 18th day and died on 20th day.
„ 2	Ditto	Paralysed on 75th day: died on 76th day. Rabies.	Rabbit inoculated subdurally with emulsion of medulla showed paralysis on 31st day and died on 34th day.
„ 3	Ditto	Died after 71 days Probably not Rabies.	Two rabbits inoculated subdurally with emulsion of medulla both died from some intercurrent disease.

Protocols.

2. Table 7. Intraperitoneal inoculation.

The virus used was the same as that which was employed in the previous series of experiments.

Each animal received intraperitoneally 5 c.c. of a 10 per cent. emulsion.

Animal.	Amount of virus.	Result.	Remarks.
Monkey 1	0·5 gramme	Died on 113th day	Rabbit inoculated subdurally with emulsion of medulla, remained well.
„ 2	Ditto	Developed paralysis on 15th day, died on 17th day.	.....
„ 3	Ditto	Developed tremor and paralysis on 16th day, died on 18th day.	.....

3. Table 8. Subdural inoculation.

The virus was the same as that which was employed in the previous series of experiments. Each animal received subdurally 0·3 c. c. of a 1 per cent. emulsion.

Animal.	Amount of virus.	Result.
Monkey 1	0·003 gramme	Paralysis on 8th day, died on 13th day.
„ 2	Ditto	Paralysis on 10th day, died on 13th day.
„ 3	Ditto	Paralysis on 9th day, died on 11th day.

D.—Table 9. Subcutaneous injection of dog virus into guinea-pigs.

The virus used was originally obtained from a dog of the street which had died of furious rabies. It had been passed through two dogs in the laboratory by subdural inoculation, both of these animals died of furious rabies, the incubation period of the last being 10 days. A one per cent. emulsion of the medulla was prepared with sterile normal salt solution. From this emulsion dilutions were made as required. Each guinea-pig received a definite quantity of virus, the amount of emulsion being in each case made up to 5 c. c. which were injected in two places in the abdominal region. Care was taken that the injections were really made into the subcutaneous tissues and that the muscles were not injured.

Protocols.

Animal.	Amount of virus.	* Result.	Remarks.
Guinea-pig 1	0·01 gramme	16 days 17 „	
„ 2	Ditto	8 „ 11 „	Five guinea-pigs out of five died of rabies.
„ 3	Ditto	10 „ 12 „	The average incubation period = 10·8 days
„ 4	Ditto	10 „ 12 „	Average interval between injection and death = 13 days.
„ 5	Ditto	10 „ 13 „	
„ 6	0·001 gramme	13 days 15 „	
„ 7	Ditto	15 „ 17 „	Four guinea-pigs out of five died of rabies.
„ 8	Ditto	17 „ 18 „	The average incubation period = 15·5 days.
„ 9	Ditto	Died on 12th day from other cause.	Average interval between injection and death = 17·5 days.
„ 10	Ditto	17 days 20 „	
„ 11	0·0001 gramme	Died on 18th day from other cause.	
„ 12	Ditto	31 days 32 „	Three guinea-pigs out of five died of rabies. Average incubation period = 24 days.
„ 13	Ditto	16 „ 18 „	Average interval between injection and death = 25·6 days.
„ 14	Ditto	Died on 8th day from other cause.	
„ 15	Ditto	25 days 27 „	

* The upper number = Incubation period.
The lower number = Interval between injection and death.

Protocols.

Animal.	Amount of virus.	*Result.	Remarks.
Guinea-pig 16	0·00001 gramme	13 days 14 ,,	
,, 17	Ditto	Died, not rabies.	Two guinea-pigs out of five died of rabies.
,, 18	Ditto	Ditto	Average incubation period = 18 days.
,, 19	Ditto	23 days 25 ,,	Average interval between injection and death = 19·5 days.
,, 20	Ditto	Died, not rabies.	

* The upper number = Incubation period.
The lower number = Interval between injection and death.

No. IV.—An attempt to immunise monkeys by means of single doses of fixed rabbit virus injected subcutaneously.

A. Six monkeys were inoculated subcutaneously in two places with 5 c.c. of a 10 per cent. emulsion of the medulla and cord of a passage rabbit, *i.e.*, total dose 5 c.c. divided into two portions. It was the 228th passage from the dog: the incubation period in the rabbit after subdural inoculation was 6 days.

Twenty-three days afterwards each monkey, and in addition two untreated ones received 0·3 c.c. of a 1 per cent. emulsion of the medulla of a passage rabbit, which had after subdural inoculation an incubation period of 6 days.

The following was the result:—

Table 10.

Animal.	Amount of virus injected subcutaneously.	Amount of virus injected subdurally.	* Result.
Monkey 1	0·5 gramme	0·003 gramme	9 days. 12 ,,
,, 2	Ditto	Ditto	7 ,, 8 ,,

* Upper figure = Incubation period.
Lower figure = Interval between subdural injection and death.

Protocols

Animal.	Amount of virus injected subcutaneously.	Amount of virus injected subdurally.	* Result.
Monkey 3	0·5 gramme	0·003 gramme	11 days. 12 ,,
,, 4	Ditto	Ditto	8 ,, 13 ,,
,, 5	Ditto	Ditto	8 ,, 10 ,,
,, 6	Ditto	Ditto	9 ,, 10 ,,
,, 7	*Nil.*	Ditto	8 ,, 13 ,,
,, 8	*Nil.*	Ditto	10 ,, 13 ,,

* Upper figure=Incubation period.
Lower figure=Interval between subdural injection and death.

B.—Six monkeys were inoculated subcutaneously with varying amounts of fixed rabbit virus. In each instance the amount was made up to 5 c. c. which were injected in two places in the abdominal region. The virus used was the 229th passage from the dog (the incubation period in a rabbit after subdural inoculation being 6 days). Twenty-three days afterwards each animal, as well as an untreated monkey, received subdurally 0·3 c. c. of a one per cent. emulsion of the medulla of a passage rabbit.

The following was the result :—

TABLE 11.

Animal.	Amount of virus injected subcutaneously.	Amount of virus injected subdurally.	* Result.
Monkey 1	0·5 gramme	0·003 gramme	7 days. 8 ,,
,, 2	0·15 ,,	Ditto	7 ,, 9 ,,

* Upper figure=Incubation period from date of subdural injection.
Lower figure=Interval between subdural injection and death.

Protocols.

Animal.	Amount of virus injected subcutaneously.	Amount of virus injected subdurally.	*Result.
Monkey 3	0·05 gramme	0·003 gramme	8 days. 9 ,,
,, 4	0·015 ,,	Ditto	8 ,, 11 ,,
,, 5	0·005 ,,	Ditto	9 ,, 12 ,,
,, 6	0·0015 ,,	Ditto	9 ,, 12 ,,
,, 7	*Nil*	Ditto	9 ,, 11 ,,

* Upper figure = Incubation period from date of subdural injection.
Lower figure = Interval between subdural injection and death.

V.—Bactericidal properties of the serum of persons taken both during the course of anti-rabic inoculations and after treatment had been completed.

A.—Experiments to show the effect of incubation at 37° C. on rabies virus.

The medulla of a fixed rabbit virus was finely emulsified in sterile normal salt solution. Two dilutions were prepared, namely, 1-200 and 1-1600. These were kept at 37° C. for varying intervals of time, 0·4 c.c. being then injected subdurally into a rabbit.

Table 12.

Animal.	Strength of dilution.	Period of time kept at 37° C.	* Result.	Remarks.
Rabbit 1	1-200	1 hour	14 days 18 ,,	
,, 2	,,	2 hours	8. ,, 11 ,,	

* Upper figure = Period of incubat on.
Lower figure = Period between injection and death.

Protocols.

Animal.	Strength of dilution.	Period of time kept at 37° C.	*Result.	Remarks.
Rabit 3	1-200	4 hours	... / 25 days	Emaciated; sub-passage gave rabies.
,, 4	,,	24 ,,	Remained well	
,, 5	1-1600	*Nil*	7 days / 12 ,,	
,, 6	,,	*Nil*	7 ,, / 10 ,,	
,, 7	,,	1 hour	Died after 26 days: not rabies.	
,, 8	,,	2 hours	24 days / 25 ,,	
,, 9	,,	4 ,,	27 ,, / 28 ,,	
,, 10	,,	4 ,,	Remained well	
,, 11	,,	24 ,,	Died after 54 days: not rabies.	
,, 12	,,	24 ,,	Died after 20 days: not rabies.	

* Upper figure=Period of incubation.
Lower figure=Period between injection and death.

B.—Experiments to test if the sera of patients taken both during the course of anti-rabic treatment and shortly after completion of treatment contain any substances which kill the rabies virus.

Blood was drawn aseptically from a vein of the arm. It was allowed to clot in a conical glass and the serum was left in contact with the clot for 24 hours.

Protocols.

The virus used was fixed rabbit virus--passage 225th-230th, which gave on subdural inoculation into rabbits an incubation period of 6 days. The medulla was used. Emulsions of different strengths were prepared with normal salt solution.

Equal quantities of the serum and of these different emulsions were mixed and the mixtures kept at room temperature for 1 or 2 hours : 0·4 c. c. of each mixture was then injected subdurally into a rabbit.

For each observation control experiments with salt solution instead of serum were made, that is to say, mixtures consisting of equal quantities of salt solution and the emulsions of different strengths were prepared, kept under the same conditions and for the same time as the serum mixtures, and 0·4 c. c. of each mixture injected subdurally into a rabbit. In some instances also control experiments with normal human serum were made in the same manner.

In all, six series of observations were made, the results of which are as follows :--

1. TABLE 13.

(*a*) Serum of patient after 9 days' treatment.
(*b*) Serum of patient after 14 days' treatment.
(*c*) Salt solution.

In each case the blood was drawn 24 hours after the inoculation.
The mixtures were left for two hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.		
	(*a*)	(*b*)	(*c*)
1 in 50	7 days* 14 „	7 days 13 „	7 days 14 „
1 in 150 . . .	7 „ 14 „	7 „ 15 „	17 „ 21 „
1 in 500 . . .	Recovered	Recovered	Recovered
1 in 1,500 . . .	Do.	11 days 17 „	Do.

* *N. B.*—In this and the succeeding tables the upper figure refers to the period of incubation of the disease after subdural inoculation of 0·4 c. c. of the mixture into a rabbit, the lower figures to the interval between injection and death.

Protocols.

2. TABLE 14.

(*a*) Pooled sera—equal parts of each—of 5 patients after 20 days complete treatment: the blood was drawn half an hour after the last injection.

(*b*) Salt solution.

The mixtures were left for two hours at room temperature.

Dilution of emulsions.	Result of inoculation into rabbit.	
	(*a*)	(*b*)
1 in 50	9 days 12 ,,	7 days 12 ,,
1 in 200	10 ,, 13 ,,	8 ,, 12 ,,
1 in 600	14 ,, 20 ,,	7 ,, 10 ,,
1 in 2,000	... 68 days	46 ,, 52 ,,

3. TABLE 15.

(*a*) Serum of patient taken one day after completion of treatment.

(*b*) Serum of patient taken 5 days after completion of treatment.

(*c*) Serum of untreated person.

(*d*) Salt solution.

Mixtures were left for 1 hour at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.			
	(*a*)	(*b*)	(*c*)	(*d*)
1 in 50 . . .	6 days 12 ,,	6 days 11 ,,	6 days 14 ,,	6 days. 10 ,,
1 in 150 . . .	7 ,, 13 ,,	7 ,, 13 ,,	7 ,, 16 ,,	8 ,, 15 ,,
1 in 500 . . .	Recovered	8 ,, 14 ,,	8 ,, 15 ,,	8 ,, 16 ,,
1 in 1,500 . .	Recovered	7 ,, 10 ,,	7 ,, 16 ,,	Recovered.

Protocols.

4. TABLE 16.

(*a*) Serum of patient taken 5 days after completion of treatment.
(*b*) Serum of patient taken 10 days after completion of treatment.
(*c*) Salt solution.

Mixtures were left for two hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.		
	(*a*)	(*b*)	(*c*)
1 in 50 . . .	10 days 16 „	Recovered	8 days. 13 „
1 in 150 . . .	8 „ 13 „	12 days 17 „	Died : not rabies.
1 in 500 . . .	Recovered	74 days	Recovered.
1 in 1,500 . . .	Recovered	Recovered	Died : not rabies.

5. TABLE 17.

(*a*) Serum of patient taken 10 days after completion of treatment.
(*b*) Serum of patient taken 14 days after completion of treatment.
(*c*) Serum of untreated person.
(*d*) Salt solution.

Mixtures were left for 2 hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.			
	(*a*)	(*b*)	(*c*)	(*d*)
1 in 50 . .	8 days 16 „	7 days 13 „	7 days 13 „	8 days. 14 „
1 in 150 . .	Recovered	8 „ 15 „	8 „ 14 „	Recovered.
1 in 500 . .	Recovered	7 „ 15 „	9 „ 15 „	Recovered.
1 in 1,500 . .	7 days 9 „	Recovered	7 „ 13 „	8 days. 11 „

Protocols.

6. TABLE 18.

(*a*) Serum of patient taken 20 days after completion of treatment.
(*b*) Salt solution.
Mixtures were left for two hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.	
	(*a*)	(*b*)
1 in 50	9 days	7 days.
	14 ,,	12 ,,
1 in 150	9 ,,	7 ,,
	14 ,,	12 ,,
1 in 500	Recovered	Recovered.
1 in 1,500	9 days	11 days.
	14 ,,	15 ,,

C.—Experiments to test if the sera of some normal animals contain substances which kill rabies virus.

Exactly the same technique was used as that which has been described for the experiments with patients' sera.

The following were the results:—

1. TABLE 19.

(*a*) Guinea-pig serum.
(*b*) Rabbit serum.
(*c*) Salt solution.
The mixtures were placed in contact for two hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.		
	(*a*)	(*b*)	(*c*)
1 in 100	8 days	8 days	7 days
	12 ,,	11 ,,	12 ,,
1 in 2,500	7 ,,	Recovered	...
	10 ,,		33 days.
1 in 5,000	Recovered	10 days	23 ,,
		11 ,,	25 ,,

2. Table 20.

(*a*) Monkey serum.
(*b*) Salt solution.
The mixtures were left for two hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.	
	(*a*)	(*b*)
1 in 100	8 days	8 days
	10 ,,	11 ,,
1 in 2,500	11 ,,	8 ,,
	13 ,,	11 ,,
1 in 5,000		8 ,,
	38 days	11 ,,

3. Table 21.

(*a*) Monkey serum.
(*b*) Rabbit serum.
(*c*) Salt solution.

In these experiments the emulsion of virus used was prepared from the medulla of a dog. It was originally obtained from a dog in nature, and it has been passed through a series of six dogs in the laboratory, the last dog after intraocular injection dying of furious rabies after an incubation period of 11½ days.

The mixtures were kept for two hours at room temperature.

Dilution of emulsion.	Result of inoculation into rabbit.		
	(*a*)	(*b*)	(*c*)
1 in 100	8 days	8 days	8 days.
	12 ,,	12 ,,	11 ,,
1 in 2,500	10 ,,	9 ,,	Died : not rabies.
	13 ,,	12 ,,	
1 in 5,000	9 ,,	11 ,,	Recovered.
	11 ,,	16 ,,	

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BY

MAJOR G. LAMB, M.D., I.M.S.

AND

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